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14. ABSTRACT

The efforts during this period divide between dendrimer preparation, peptide design and preparation, and the exploration of conjugation chemistries. Alkyne-functionalized dendrimers over many generations have been successfully prepared. Therapeutic peptides have been designed, prepared, and submitted to the co-investigators for evaluation. Two conjugation strategies for ligating the therapeutic peptide to the dendrimer platform are being investigated.

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none

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INTRODUCTION: The goals of this multiyear effort focus on the synthesis and characterization of dendrimers that vary in size from generation 3-7 with a functional alkyne core (0-6 mo). This core will be used to install a chelate group for diagnostic medical imaging (4-12 mo). The surface groups will be surveyed for optimal behavior in the therapeutic application (9-18 mo). Therapeutic peptides will be designed and installed (12-24 mo), and the cycle will be iterated over the period of the project with feedback from collaborators at the University of Texas Southwestern Medical Center.

BODY: The organization follows the individual items in the SOW with each constituting an independent, numbered section.

1. **Synthesis and characterization of dendrimers that vary in size from generation 3-7 with a functional alkyne (0-6 mo).** This work is almost wholly complete. Building from efforts that were reported simultaneously with the award of the grant, odd generation dendrimers have been prepared through generation 13. [1] These materials bear an alkyne core. The descriptor "functional" refers to our ability to react this alkyne using so-called "click" chemistry to install a diagnostic group of interest. We have established that the click reaction works on low generation materials through generation 5. We are focusing on these materials for a variety of reasons that include ease of preparation and our desire to transition to more challenging elements of the SOW. In addition, independent efforts have accelerated this synthesis and also increased the scale of process thus allowing more dendrimer to be created. It is foreseeable that while installation of the alkyne has been successful, the descriptor "functional" based on its amenability to reaction may not be so. Computational models suggest that generation 6 and 7 dendrimers may present significant crowding that precludes chemical reaction. These targets are still accessible, hypothetically, by installing the diagnostic imaging group on the generation 5 dendrimer and iterating through the dendrimer synthesis to 6 and 7. This strategy has not been employed largely because the alkyne is chemically inert to the existing reaction conditions employed during dendrimer synthesis. Delaying installation of the diagnostic imaging group cuts down on the amount of the group required to be used due to losses that are intrinsic to any multistep synthesis as well as the freedom to select any number of different groups as imaging agents at the very end of the process.
2. **Installation of the chelate group for diagnostic medical imaging (4-12 mo).** A very small sample of chelate was obtained from the Sun group at UTSW, but the purity and amount was insufficient to perform meaningful conjugation chemistry at the site of the alkyne. We are

currently making a much less complex model that mimicks the functional and chemical characteristics of this material for exploratory studies. Emphasis instead focuses on SOW item #3.

3. **Exploration of surface groups to promote desired behavior (9-18 mo).** We have established strategies for making the desired platform water soluble with anionic, cationic or neutral charge. The therapeutic peptides of interest present significant challenges to solubility. The lead compound containing a hydrophobic proline-rich domain and hydrophilic polycationic domain that was advanced by UTSW presents solubility problems and requires administration with a cosolvent, DMSO. The dendrimer should alleviate some of these challenges.
4. **Therapeutic peptides will be designed and installed (12-24 mo).** This effort was initiated early based on the UTSW observations of challenges to solubility. To this end, multiple therapeutic peptides were prepared to examine how the domain order impacts solubility and activity. While solubility was not affected, domain order did impact bioactivity. This important finding shapes our pursuit for the next period. Appropriately ordered therapeutic peptides were attached to the dendrimer and solubility is currently being probed as a function of auxillary surface groups and number. Additionally, the number of peptide domains is being varied. This phase will occupy most efforts for this upcoming period. In addition, efforts to look at labile linkers designed to release the therapeutic peptide have advanced, and the results will be submitted for publication shortly.

KEY RESEARCH ACCOMPLISHMENTS:

- Functional dendrimers of generation 3-5 have been prepared.
- Imaging agents have been installed using the proposed chemistry
- Therapeutic peptides have been designed and characterized such that bioactive constructs can be pursued
- Preparation of these constructs has identified the next set of challenges: solubility and surface group selection

REPORTABLE OUTCOMES: No reportable outcomes to date.

CONCLUSION: Efforts ongoing are considered to be in-line with milestones identified in the original SOW with key barriers to the identification of a theranostic overcome, and existing challenges well articulated. No deviation from the proposed SOW is requested.

REFERENCES:

[1] Synthesis of Large Dendrimers with the Dimensions of Small Viruses**.** Lim, J.; Kostiainen, M.; Maly, J.; da Costa, C.P.; Annunziata,O.; Pavan, G.M.; Simanek, E.E. *J. Am. Chem. Soc.* **2013**, *135*, 4660-4663.

APPENDICES: None included.

SUPPORTING DATA: None included.